Toxigenicity of *Fusarium proliferatum* and other *Fusarium* Species Isolated from Corn Ears in Minnesota

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ABSTRACT

Abbas, H. K., Mirocha, C. J., Kommedahl, T., Burnes, P. M., Meronuck, R. A., and Gunther, R. 1988. Toxigenicity of *Fusarium proliferatum* and other *Fusarium* species isolated from corn ears in Minnesota. Phytopathology 78:1258-1260.

Eighty-one isolates of *Fusarium* species isolated in 1986 from naturally contaminated ears of corn (*Zea mays* L.) in southern Minnesota were identified as *F. proliferatum* (11 isolates), *F. subglutinans* (23 isolates), *F. graminearum* (20 isolates), *F. moniliforme* (19 isolates), and *F. oxysporum* (eight isolates). When these isolates were single-spored, grown on a sterile rice substrate, and fed to rats, *F. proliferatum* caused hemorrhage (five isolates), diarrhea (one isolate), and death (nine isolates); *F. subglutinans* caused death (eight isolates); *F. graminearum* caused uterine enlargement (14 isolates), hemorrhage (four isolates), and death (nine isolates); *F. moniliforme* caused hemorrhage in various organs (13 isolates), diarrhea (three isolates) and death (16 isolates); and *F. oxysporum* caused hemorrhage (one isolate) and death (one isolate). All 81 isolates caused rats to gain less than those fed control diets. This is the first report of *F. proliferatum* causing organ hemorrhage as well as death.

Moldy grain has been observed since antiquity, but the toxicity of moldy ears of corn (*Zea mays* L.) to farm animals was not considered seriously before 1955 in the United States (7). Interest in mycotoxicoses started about the time *Fusarium* species were being studied extensively but before species identification had reached modern standards. *F. moniliforme* Sheldon has been the most widely reported *Fusarium* species in kernels (13) and is isolated from nearly 100% of the kernels in a given seed lot. Next in frequency of isolation is *F. subglutinans* (Wr. & Reink.) Nels., Toussoun and Marasas, followed by *F. graminearum* Schwabe, and to a much less extent, *F. tricinctum* (Cda.) emend. Snyd. & Hans. (probably *F. sporotrichioides* Sherb.), *F. oxysporum* Schlect. emend. Snyd. & Hans., *F. equiseti* (Cda.) Sacc. sensu Snyd. & Hans., *F. solani* (Mart.) App. and Wr. emend. Snyd. & Hans., and *F. semitectum* Berk. & Rav. Marasas et al (16), among others (14,15,22), have reported that all of these and additional species of *Fusarium* are toxigenic to a variety of farm animals.

Many accounts of toxigenic species of *Fusarium* have been described throughout the world. For example, Mirocha (17) reported that *Fusarium* species were involved in toxicological problems in human and farm animals. The trichothecene mycotoxin T-2 has been reported to be responsible for the death of lactating cows feeding on moldy corn (4,12). Equine leucoencephalomalacia in horses has been associated with the presence of *F. moniliforme* in corn grain that was part of the animals' diet (5,21,23,24). Many isolates of *Fusarium* species obtained from foodstuffs and soil from various parts of the world proved toxic to human and mouse fibroblast cells and to rats, according to Abbas et al (3). The Kashin-Beck disease of humans was attributed by Yang et al (26) to the presence of *F. oxysporum* in corn. From freshly harvested grain in southern Japan, Yoshizawa et al (27) isolated 106 strains of *F. graminearum* that were found to be toxigenic. In fact, Marasas et al (16) list more than 200 toxigenic strains in 20 *Fusarium* species.

Unusually wet weather in Minnesota in 1986 near harvest time favored the development of considerable fungus growth on ears of corn in the field. Samples from 10 counties were collected for assay of fungi on ears and for testing their toxicity to animals. Abbas et al (2) identified the species of *Fusarium* as well as the mycotoxins present in the infected ears. However, they did not determine the toxigenicity of these isolates to animals. Thus, our objective was to test isolates of the five species of *Fusarium* from moldy corn for toxicity to rats when fed a diet containing fungus-contaminated rice, with special interest in *F. proliferatum* (Matsushima) Nirenberg (due to the dearth of information on this recently described species), and *F. subglutinans* (because of the incomplete and ambiguous reports of toxigenicity of this species).

MATERIALS AND METHODS

The sources of *Fusarium* cultures used in this study were described earlier by Abbas et al (2), and were obtained principally from 10 counties in southern Minnesota on ears of corn in which at least 10% of a given ear (without the husk) was visibly moldy in the field. Species were identified as described by Nelson et al (20). Isolates were single-spored as reported by Nelson et al (20) with modification (C. E. Windels, unpublished). On petri dishes containing 8–10 ml of 2% water agar, a small agar square of an actively sporulating culture was transferred to 5 ml of sterile distilled water in vials. The conidial suspension was agitated for 5 sec with a vortex mixer. With a flame-sterilized wire loop, one loopful of the suspension was streaked over the agar surface to form one side of a triangle and repeated to complete the other two sides of the triangle. Finally, the loop was flame sterilized again and streaked over the entire agar surface, and at the same time, the dish was rotated so spores would be distributed uniformly. After 24 hr, dishes were examined under a dissecting microscope, and single germinating spores were transferred to carnation-leaf agar or acidified potato-dextrose agar. Stock cultures were maintained on silica gel, described by Windels et al (25), but stored at −15 C.

Fusarium species to be tested for toxigenicity were cultured on an autoclaved rice medium, described as a modification of the procedure developed by Eugenio et al (8) and reported also by Abbas and Mirocha (3). In brief, 200 g of long-grain parboiled rice (Uncle Ben's, Houston, Texas) and 120 ml of distilled water were allowed to stand for 1 hr in a 1-L flask, then autoclaved for 60 min at 121 C twice with a 24-hr interval. Flasks containing this medium were inoculated with *Fusarium* species and incubated at room temperature (24 C) for 2 wk, during which flasks were shaken daily for the first few days; these flasks were then transferred to an incubator at 10–12 C for 2 wk. The mycelial mass and substrate were disbursed onto a screen-bottom tray and allowed to air dry in a ventilated hood. When dry, this inoculated substrate was ground to the consistency of flour and used to feed rats (20-day-old virgin female Sprague-Dawley rats, available from Bio-Lab Corp., St. Paul). The rats were housed in individual cages and fed a 1:1 mixture (v/v) of the inoculated rice and a complete rat diet (Ralston-Purina). The complete rat diet consisted of the following ingredients: mineral, 175 g; sucrose, 525 g; vitamins, 50 g; corn oil, 200 ml; casein, 600 g; and corn grain, 950 g. This was mixed until corn oil and other ingredients were well distributed. Then 2,500 g of ground corn grain was added and mixed thoroughly to give 5,000 g. Rats were weighed at the start and end of the experiment, and three rats per *Fusarium* species were tested. Two controls were used in which rats were fed a complete rat diet or a 1:1 mixture of ground, autoclaved rice and a complete rat diet. After 5 days, the rats were killed by cervical dislocation and examined for gross pathological changes in organs and tissues.

RESULTS

Of 81 cultures of *Fusarium* species, 11 were *F. proliferatum*, 23 were *F. subglutinans*, 20 were *F. graminearum*, 19 were *F. moniliforme*, and eight were *F. oxysporum* (Table 1). These 81 isolates representing five species of *Fusarium* comprised 52 isolates that were toxic and 43 that were lethal to rats fed a diet of rice inoculated with *Fusarium* species. Of 11 isolates of *F. proliferatum* grown on a rice substrate and fed to rats, four caused hematuria (blood in bladder), one caused diarrhea, one caused internal hemorrhage, and nine caused death.

Of 23 isolates of *F. subglutinans*, eight caused death in rats; none of the remaining isolates produced symptoms in tissue (Table 1). Of 20 *F. graminearum* isolates, 14 caused uterine enlargement, four caused hematuria, and nine caused death; two isolates did not elicit symptoms. Of 19 isolates of *F. moniliforme*, two caused intestinal hemorrhage, two produced rectal hemorrhage, nine caused hematuria, three produced diarrhea, and 16 caused death; five isolates elicited no symptoms. Of eight isolates of *F. oxysporum*, one produced hematuria and death. All 81 isolates caused decreased weight gains relative to control diets, and the control diets had no visibly deleterious effects on rats.

DISCUSSION

The five species of *Fusarium* that we isolated from ears of corn have been reported before by us (2) and by others (7,13,17). *F. graminearum* and *F. moniliforme*, especially, have been widely reported on corn grain. *F. proliferatum* and *F. subglutinans* have had a different history in that both species often have been cited in the literature as *F. moniliforme*, partly because *F. proliferatum* was only recently distinguished from *F. moniliforme* (10) and because *F. subglutinans* has been included as a variety or cultivar of *F. moniliforme*. *F. subglutinans* may have been identified as *F. oxysporum* if the observer had not looked for the presence or absence of polyphialides especially when chlamydospores were not readily apparent. By using single-spore cultures of these species growing on a rice substrate and feeding the ration and culture substrate to rats, it was possible to get better information on the toxigenicity of these five *Fusarium* species with more recent taxonomic distinctions.

Toxicological information on *F. graminearum*, *F. moniliforme*, and *F. oxysporum* is fairly extensive and is summarized by Marasas et al (16). Less is known about *F. subglutinans* probably because of confusion regarding its taxonomy. Still less is known about *F. proliferatum* because of its relatively recent description as a taxon, and we report its toxicity to rats for the first time in this paper. Nine of 11 isolates of *F. proliferatum* caused death of rats, and four caused hematuria either with accompanying symptoms of diarrhea or intestinal hemorrhage, as well as being lethal.

Also significant is the finding that eight of 23 isolates of *F. subglutinans* accounted for death in rats, but 15 isolates caused no visible toxic effects, although weight gains were depressed in rats feeding on the rice medium inoculated individually with all 23 isolates. As pointed out by Marasas et al (16), records on toxigenicity are confusing because of possible misidentification of species. It is apparent also that not all isolates of a given species behave in the same way.

All five species of *Fusarium* that we found have been reported to produce trichothecenes, except for *F. moniliforme* (16), which produces the nontrichothecene moniliformin. All *Fusarium* species except *F. graminearum* produced detectable amounts of moniliformin (Abbas et al unpublished). Fourteen isolates of *F. graminearum* caused uterine enlargement, an indication that zearalenone was produced (18,19), and this toxin was detected in moniliformin (Abbas et al, *unpublished*). Fourteen isolates of *F.* mycotoxins such as T-2 and diacetoxyscirpenol have been reported to cause hemorrhage, diarrhea, and death in animals (9,17,23), and moniliformin is lethal at high concentrations to mice, rats, and sheep (6,11,14,15). Research by Abbas and Mirocha (1) has identified a new hemorrhagic factor (wortmannin) produced by *Fusarium* species, and its possible presence in these feeds may explain in part the toxigenicity of these specific isolates.

Twenty-three of the 81 isolates of *Fusarium* caused hemorrhage

TABLE 1. Toxicity of *Fusarium* isolates to rats fed at 1:1 mixture of fungal culture (on rice) and a complete rat diet

Fusarium species	No. of isolates	No. of isolates causing[a]				Range of food consumed (g)
		Death	Toxic signs	Wt. loss	Wt. gain	
Control 1[b]	···	0	0	0	0	40
Control 2[b]	···	0	0	0	0	41
F. graminearum	20	9	17	11	0	3–31
F. moniliforme	19	14	12	5	0	2–29
F. oxysporum	8	1	1	2	5	14–38
F. proliferatum	11	9	4	2	0	4–25
F. subglutinans	23	8	6	1	14	2–37

[a]Toxic signs are uterine enlargement, hematuria, intestinal and rectum hemorrhage, and diarrhea. Results based on three rats per treatment.
[b]Control 1 = mixture of 1:1 autoclaved rice and complete rat diet; control 2 = complete rat diet only.

of various organs, four of the isolates caused diarrhea, and 42 isolates caused death of rats. Because all of the symptoms described were found in this study, there probably was one or more toxins produced by the isolates in the rice medium. This medium was selected because the greatest toxicity of *Fusarium* isolates had been demonstrated by use of this rice medium (3,8). Some of the toxins from these corn samples were identified by Abbas et al (2), and this report is a continuation of work demonstrating that *Fusarium* species not only produce toxins in ears of corn in the field, but also that these fungi produce toxins in the laboratory.

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